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COMPARATIVE STUDY ON PHYSICOCHEMICAL VARIATION OF *MICROSTYLIS WALLICHII:* A DRUG USED IN AYURVEDA

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ABSTRACT

The present study is intended primarily to confirm harmonization with respect to quality of the drug. In the lack of proper production and supply system and the increasing demands of herbal drug are the major factors promoting the practices of adulteration and substitution. Therefore, the standardization of the herbal drugs is essential for assuring the therapeutic efficacy of the herbal drugs. The comparative studies were carried out to evaluate the pharmacognostical as well as physico-chemical standards of *Microstylis wallichii* with emphasis on TLC fingerprinting of the drug for chemical identification. Samples were procured from two diverse vicinities to determine the quality variations varied with the place of collections. The physiological and non-physiological contaminations were also reported.

KEYWORDS- *Microstylis wallichii*, Physico-chemical studies, Standardization, TLC studies, Ash-value, Herbal drugs

INTRODUCTION

Microstylis wallichii Lindl is a Rasayana and belongs to the "*Ashtverga.*'. It constitutes a group of eight drugs, which form an important constituent of a number of *Ayurvedic* preparations with the help of which wonderful cures have been claimed, but these drugs have not been properly identified even today. These drugs are known in Sanskrit as *Jiwak*, *Rishbhak (Microstylis wallichii* Lindl*)*, *Mahameda*, *Meda*, *Kakoli*, *Kharkakoli*, *Ridhi* and *Bridhi* (Chunekar, 1982). Extremely vague descriptions about these are available in the *Ayurvedic* literature and it has so far not been possible to ascertain what particular botanical plants actually these are. The result is that all sorts of plants are currently being used as "Ashtaverg" (Chopra *et al.*, 1956). *Microstylis wallichii* Lindl. var. bilbo (Lindl) Hook (family: *Orchidaceae*) has also been reported by another vernacular name *Malaxis accuminata* D. Don (Rastogi and Mehrotra, 1993). Morphological and histological characters of the drug have been reported (Bhatnagar and Shant, 1966).

The extensive literature survey of the plant revealed that the no research work dealing with the isolation and characterization of phytochemicals and their pharmacological activities of *Microstylis wallichii* Lindl has been carried out so far. However, one sterol namely β-sitosterol and an alcohol identified as cetyl alcohol, two sugars namely glucose and rhamnose and five basic compounds one of them being cholin were reported from the *Microstylis wallichii* Lindl (Bhatnagar *et al*., 1970). Limonene, eugenon, citronellal, 1,8-cineole, piperitone and p-cymene were reported to occur in *Malaxis accuminata* by thin layer chromatographic separation (Gupta *et al*., 1978).

In this study we aimed to provide more information and scientific validation of the *Microstylis wallichii* Lindl for the acclaimed medicinal use and to determine the quality of the drug varied with the place of collection from two diverse vicinity.

MATERIALS AND METHODS

The tubers of *Microstylis wallichii* Lindl were supplied by Regional Research Institute, Tarikhet (Ranikhet), Uttranchal and also purchased from herbal drug supplier, Lucknow U.P (Table 1). Microtome section were taken, stained and mounted following the usual plant micro-techniques (Kay., 1938 ; Johnsen., 1940) and representative diagram were sketched through Lucida camera. For study of isolated cells and tissues, small pieces of tubers were macerated with schult's fluid, washed and mounted in glycerin. Physio-chemical test were carried out adopting, standard procedure (Trease and Evan., 1983 ; Kokate *et al*., 2001). Ash-value, solubility (cold percolation and graded extraction) in the various solvents such as petroleum ether, alcohol (95%) and water values, screening of thin layer chromatography, effect of different chemical reagents and florescence analysis under ultra-violet radiation which are considered to be immense help in detection of adulterants were also carried out (Chase and Pratt., 1949 ; API, 1999 ; PSAF, 1987 ; Winton and Winton., 2001). Qualitative for identification of phyto-constiuents like alkaloids, steroids terpenoids, phenols, tannins, saponins and flavinoids etc, were also carried out (Kokate *et al*., 2001).

TLC Analysis

Various extracts of *Microstylis wallichii* Lindl were subjected to TLC on silica gel-G (manually coated on glass plate in laboratory). Various combinations of the solvents of different polarity were adjusted to found out the suitable TLC pattern of the drug. The resolution pattern was detected in iodine-chambers and Lieberman-reagent. They are summarized in Table 7.

RESULTS

Description of Tubers

The tubers were 3-6 cm long, 0.5-1.0 cm thick and have a fibrous root, which arises from the base. Odour was characteristic with no taste; surface was smooth and brown, yellowish in colour. They were covered externally with white papery membrane. Organoleptic identification of *Microstylis wallichii* Lindl. have been summarized in Table 2.

Powder Description

Microscopic examination of the powder showed calcium oxalate crystals of various shapes and sizes, parenchymatous cells single or in groups containing yellowish brown pigments. Fibres, vessels and trachoids were single or in groups. No fungus had developed during storage.

Transverse section. of *Microstylis wallichii* Lindl was circular in outline. Epidermal cells were barrel shaped having cuticle in outline. Below epidermis, large ground tissue consisting of parenchymatous cells. Parenchymatous cells towards epidermis were smaller in size while towards center they were bigger. Parenchymatous cells had sufficient inter-cellular spaces. Vascular bundles were scattered in ground tissue. Phloem was encircled by xylem. Numerous mucilage canals were present in ground tissue. Vascular elements showed scalariform and spiral thickening (Figure 1).

DISCUSSION

The powder of the drug *Microstylis wallichii* Lindl was non-hygroscopic in nature. The qualitative analysis of both the samples (Tarikhet and market) was showed that the most of the major plant metabolites are common, except for alkaloids and flavonoides, which were negative in the market sample (Table 3). The difference in foaming index of both the samples of *Microstylis wallichii* Lindl (Tarikhet<1000 and Market 333) indicate that the foaming ability of the drug decoction is governed by the nutrient and chemical nature of the soil from which the drug is collected (Table 4).

Comparative lower value of swelling index (Tarikhet 1.92, Market 1.12) reveals that the Tarikhet sample of *Microstylis wallichii* Lindl having appreciably more amounts of mucilage, pectin and hemicellulose compared to the market sample (Table 4).

The comparison of total ash value suggests that the total physiological and non-physiological ash is higher in Tarikhet sample compared to the market sample (Table 4). But the non-physiological ash (acid insoluble

value), clearly indicate that the market sample is contaminated with silica or siliceous matters. The chemical assay of *Microstylis wallichii* Lindl is yet to be instituted.
The variation in extractable matter in various solvents is suggestive of the fact that the formation of bioactive principles of medicinal plants is influenced by number of intrinsic and extrinsic factors, leading to strange qualitative and quantitative changes making such plants totally unfit for prescribed purpose even of same species. This is highlighting the importance of chemical mapping of the drug species.

Behavior of drug powder in different reagents was observed under ordinary light and UV radiation (254 and 366nm) (Tables 5&6). None of reagent shows the diagnostic colour reaction under either ordinary or UV radiation. The TLC patterns were recorded and the results are tabulated (Table-7). Following the instruction of Indian Herbal Pharmacopoeia (IHP, 1999) TLC pattern of purified methanol fraction (the coloring matter was removed with the help of activated charcoal) was obtained. The preliminary TLC studies revealed that the solvent system was ideal and gave the well-resolved spot of the fractions. The chemical mapping of various extracts of *Microstylis wallichii* Lindl was carried out by means of thin layer chromatography. Thus the quality of the wild crafted drug *Microstylis wallichii* Lindl varied with the place of collection. However, this conclusion required further experimental elaboration.

Therefore, more useful work for purification, isolation and characterization on bioactive compound of *Microstylis wallichii* Lindl are required.

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Figure 1

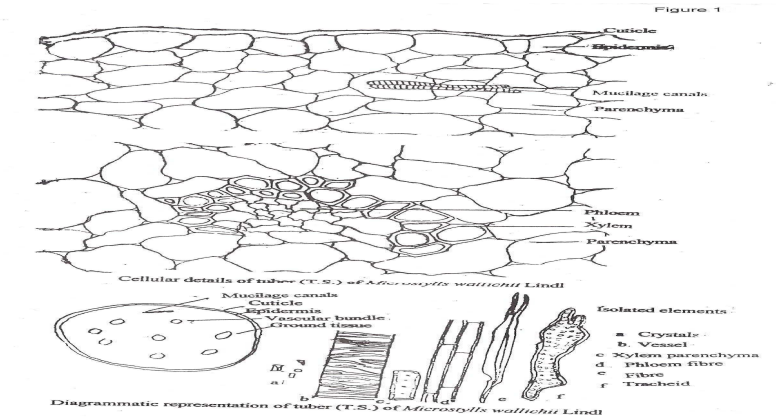

Table 1: The topographical details of collection of *Microstylis wallichii* Lindl.

Place of collection	Supplied by CRI, Tarikhet (Uttaranchal)	Market Sample*
Time of collection	NP	-
Location	NP	-
Climate	Subtropical region, Hill area.	-
Rainfall average	50-70 cm (Annual)	-

NP- Not provided by the genuine drug supply unit.
* No information regarding the origin of sample.

Table 2: Organoleptic identification of *Microstylis wallichii* Lindl.

Parameters	Observations	
	Tarikhet sample	Market Sample
Botanical		
Sensory Evaluation Visual macroscopy Touch Odour Taste Colour	 Curved Rough Characteristic Light sweet Green to brown	 Curved Rough Characteristic Tasteless Yellowish to brown
Foreign Organic Matter	No adulterants have been found	No adulterants have been found

Table 3: Qualitative analysis of plant metabolites (primary and secondary both) of *Microstylis wallichii* Lindl.

S. No	Phytochemicals	*Microstylis wallichii* Lindl (Tarikhet sample)	*Microstylis wallichii* Lindl (Market sample)
1.	Alkaloid	+ve	-ve
2.	Carbohydrate	+ve	+ve
3.	Flavonoid	+ve	-ve
4.	Protein	-ve	-ve
5.	Resin	+ve	+ve
6.	Saponin	+ve	+ve
7.	Starch	+ve	+ve
8.	Steroids	+ve	+ve
9.	Tannin	+ve	+ve
10.	Triterpenoids	-ve	-ve

Table 4: Physico-chemical Parameters of Microstylis *wallichii* Lindl.

S. N0.	Parameters	Observations	
		Tarikhet sample	Market Sample
1.	Physicochemical		
	Ash Values (% w/w)		
	a) Total Ash Value b) Acid Insoluble Ash c) Water Soluble Ash	6.25 ± 0.50 3.00 ± 0.03 4.00 ± 0.43	4.80 ± 0.39 3.75 ± 0.04 4.00 ± 0.43
	Extractive values (% w/w)		
	Cold percolation method PE (40-60°) EtOH (95%) H_2O	 4.66±0.3 4.80±0.3 6.25±0.5	 9.50 ± 0.4 4.66 ± 0.8 18.3 ± 1.2
2.	*Soxhlet graded extraction method* PE (40-60°) EtOH (95%) H_2O Moisture content (Tarikhet sample fresh; Market sample -air dried) Volatile Oils (%v/w) Pharmacological Swelling Index Foaming Index	 6.25 ± 0.4 4.75 ± 0.4 11.25 ± 0.9 36.49 ± 1.18 (% w/w) By Hot air Oven method 35.12 ± 0.98 (%v/w) By Azeotropic method 0.54 ± 0.28 1.92 ± 0.14 more than 1000	 8.00±0.5 5.00±0.7 10.00±0.9 6.65± 0.50 (% w/w) By Hot air Oven method 7.02 ± 0.42 (%v/w) By Azeotropic method ND 1.72±0.20 333

Values presented are mean of triplicate (Mean ± S. d.), ND-: Not detected

Table 5: Behavior of *Microstylis wallichii* Lindl (Tarikhet) powder with different reagents observed under ordinary light and UV-radiation.

S.NO.	Interaction of *Microstylis wallichii* Lindl (Tarikhet Sample) powder with different reagent	Colour produced under ordinary light	Colour produced under *UV-radiation*	
			Short (254nm) wavelength	Long (366nm) wavelength
1.	Drug (P) as such	Grey	Blue	Violet
2.	P + Nitrocellulose in amyl acetate	Brown	Pink	Green
3.	P+1N.NaOH in water	Light Brown	Violet	Light Green
4.	P+1N.NaOH+ Nitrocellulose in amyl acetate	Dark Brown	Brown	Dark Green
5.	P+1N.HCL+Nitrocellulose in amyl acetate	Brown	Dark Brown	Brown
6.	P+1N.NaOH in Methanol	Grey	Red	Green
7.	P+50%KOH	Brown	Pink	Brown
8.	P+1N.HCL	Light Brown	Violet	Dark Brown
9.	P+50%H_2SO_4	Brown	Brown	Pink
10.	P+50%HNO_3	Violet	Dark Brown	Brown
11.	P+Conc.HNO_3	Brick Red	Brown	Light Brown
12.	P+ Acetic acid	Grey	Light grey	Violet
13.	P+Conc. H_2SO_4	Light Brown	Dark red	Brown
14.	P+ Iodine water	Brown	Brown	Dark Brown

Table 6: Behaviour of *Microstylis wallichii* Lindl (Market sample) powder of pseudobulb with different reagents observed under ordinary light and UV-radiation.

S.NO.	Interaction of *Microstylis wallichii* Lindl (Market Sample) powder with different reagent	Colour produced under ordinary light	Colour produced under *UV-radiation*	
			Short (254nm) wavelength	Long (366nm) wavelength
1.	Drug (P) as such	Grey	Blue	Violet
2.	P+ Nitrocellulose in amyl acetate	Brown	Pink	Green
3.	P+1N.NaOH in water	Light Brown	Violet	Light Green
4.	P+1N.NaOH+ Nitrocellulose in amyl acetate	Dark Brown	Brown	Dark Green
5.	P+1N.HCL+Nitrocellulose in amyl acetate	Brown	Dark Brown	Brown
6.	P+1N.NaOH in Methanol	Grey	Red	Green
7.	P+50%KOH	Brown	Pink	brown
8.	P+1N.HCL	Light brown	Violet	Dark Brown
9.	P+50%H_2SO_4	Brown	Brown	Pink
10.	P+50%HNO_3	Violet	Dark Brown	Brown
11. P+Conc. H_2SO_4		Brick Red	Brown	Light Brown
12.	P+ Acetic acid	Yellow	Grey	Light yellow
13.	P+Conc. H_2SO_4	Light Brown	Dark red	Brown
14.	P+ Iodine water	Brown	Brown	Dark brown

Table 7: Thin Layer Chromatographic pattern of extract of *Microstylis wallichii* Lindl (pseudobulb) of Tarikhet and Market samples.

S.N0.	Stationary Phase	Solvent System	Loading Extract	Visualisation/ Detection	R_f Value (R_f x 100)
1.	Silica gel-G	Toluene:EtOAc:MeOH:AcOH (20:4:1:2drop)	MeOH (graded) Market	I_2	35.7, 57.1, 71.4, 85.7
2.	Silica gel-G	Toluene:C_2H_5O C_2H_5 (3:1)	MeOH (Cold) Market	I_2	64.2, 75.0, 86.4
3.	Silica gel-G	C_6H_6:Toluene:MeOH:AcOH(15:5:5:2drops)	MeOH (graded) Market	I_2	33.3, 44.4, 53.3, 56.0
4.	Silica gel-G	Hexane:Acetone:MeOH(2:2:1)	MeOH (graded) Tarikhet	LB	23.0, 40.0, 55.0, 69.0
5.	Silica gel-G	MeOH: $CHCl_3$:Hexane (1:1:4)	MeOH (Cold) Tarikhet	LB	10.7, 25.0, 42.0, 75.0, 67.2, 72.6, 82.0, 85.0
	Silica gel-G	……..do…..	MeOH (graded) Tarikhet	…do..	17.0, 35.7, 46.0, 57.0, 71.0
6.	Silica gel-G	PE: C_6H_6 (1:2)	PE (Cold) Market	LB	22.0, 33.0, 48.0, 62.0
	Silica gel-G	……..do…….	PE (graded) Market	LB	33.0, 37.0, 77.0, 81.4
7.	Silica gel-G	Heptane:n-butanol: MeOH(3:2:1)	MeOH (graded)Market	I_2	15.3, 30.7, 53.0, 53.8,, 76.9
	Silica gel-G	…….do…….	(Tarikhet Sample)	..do…	15.3, 30.7, 53.8, 73.7
8.	Silica gel-G	Hexane:Acetone:AcOH (20:10:2drop)	MeOH (graded)Market	I_2	26.6, 36.6, 66.6
	Silica gel-G	…….do…….	(Tarikhet Sample)	..do…	13.3, 20.0, 33.8, 54.7
9.	Silica gel-G	Heptane:EtOH(2:1)	PE (graded)Market	I_2	37.5, 50.0, 62.5, 66.6, 90.0
	Silica gel-G	…….do…….	(Tarikhet Sample)	..do…	37.5, 41.6, 50.0, 62.5, 90.0
10.	Silica gel-G	Hexane: $CHCl_3$: MeOH(4:1:1)	PE (graded) Market	I_2	57.6, 73.0, 84.6, 96.0
	Silica gel-G	…….do…….	(Tarikhet Sample)	..do…	57.6, 76.0, 84.6, 96.0

MeOH-: Methanol, EtOH-:Ethanol, PE-:Petroleum ether, $CHCl_3$-:Chloroform , C_6H_6-:Benzene, EtOAc-:Ethyl acetate, $C_2H_5OC_2H_5$-:Diethyl ether

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